Conformational Analysis and Structure–Activity Relationships of Selective Dopamine D-1 Receptor Agonists and Antagonists of the Benzazepine Series

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Comprehensive conformational analysis using molecular mechanics calculations (MM2(85)) has been carried out for the potent and selective dopamine D-1 receptor agonist 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (1; SK&F 38393), the antagonist 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (8; SCH 23390), and several analogues, including conformationally constrained ones. Calculated conformational energies have been related to pharmacological and biochemical data in an attempt to identify the biologically active conformations of 1 and 8. It is concluded that the most probable receptor-bound conformation in both cases is a chair conformation with an equatorial phenyl ring and for 8 an equatorial *N*-methyl group. It is suggested that the orientation of the phenyl ring in the receptor-bound molecule does not deviate in terms of dihedral angles by more than about 30° from the preferred phenyl group rotamer in which the planes of two aromatic rings are essentially orthogonal.

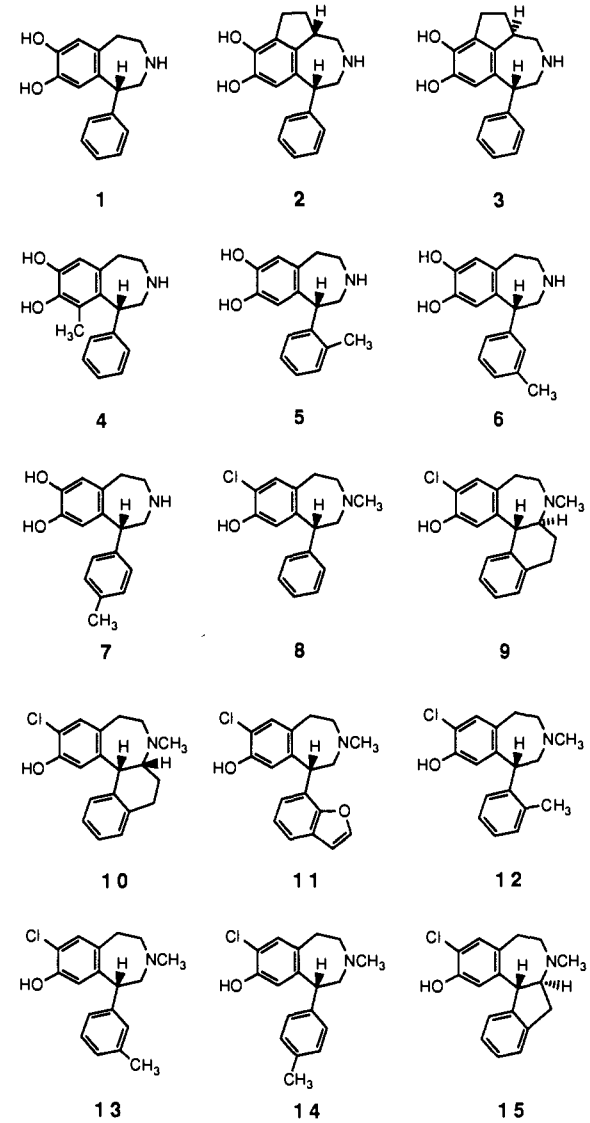
Dopamine receptors are divided into two subpopulations, D-1 and D-2, based on their biochemical characteristics.[1] Selective agonists and antagonists are known for both subtypes of receptors, but the molecular factors which are responsible for the selectivity are poorly understood.

7,8-Dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SK&F 38393 (1), Chart I) is a prototype of potent and selective D-1 receptor *agonists*. This compound displays a high degree of enantioselectivity and the activity resides almost exclusively in the *R* enantiomer.[2,3] 7-Chloro-3-methyl analogue 8 (SCH 23390, Chart I) is a very potent and selective dopamine D-1 receptor *antagonist*.[4] Also in this case the *R* enantiomer is by far the most active one.[5-7]

Compounds 1 and 8 may adopt a number of different conformations. As shown in Figure 1, the tetrahydroazepine ring may adopt chair (**a**–**d**), twist (**e**–**h**), and boat (**i**–**l**) conformations. The phenyl ring and the nitrogen substituent may be in an axial or in an equatorial position. In addition, rotation about the bond connecting the phenyl and the seven-membered rings results in different angles between the planes of the two aromatic rings. Several attempts to identify the conformation of 1 which is responsible for its biological effect ("the biologically active conformation") have been reported, but so far it has not been possible to reach an unambiguous solution to this basic problem, which is of decisive importance for progress toward an understanding of dopamine D-1 receptor selectivity and for the design of new D-1 selective compounds.

Dandridge et al.[8] suggest that the biologically active conformation of the tetrahydroazepine ring in 1 is a quasichair conformation with an pseudoaxial phenyl ring essentially as in Figure 1, structures **c** and **d**. This suggestion is based on a conceptual model for the dopamine receptor proposed by McDermed[9] and on a structural comparison between the active enantiomers of 1 and 3′,4′-dihydroxynomifensine.[8] Weinstock et al. used ethano-bridged derivatives of 1 to probe this suggestion.[10] However, a clear-cut conclusion could not be reached. The pharmacological and biochemical data, in conjunction with conformational analysis, could be interpreted in terms of a chair conformation of the tetrahydroazepine ring with an equatorial phenyl ring (Figure 1, structures **a** and **b**), as well as in terms of a twist conformation with an axial phenyl ring (Figure 1, structures **e** and **f**). Van de Waterbeemd et al. employed a twist conformation with an

Chart I



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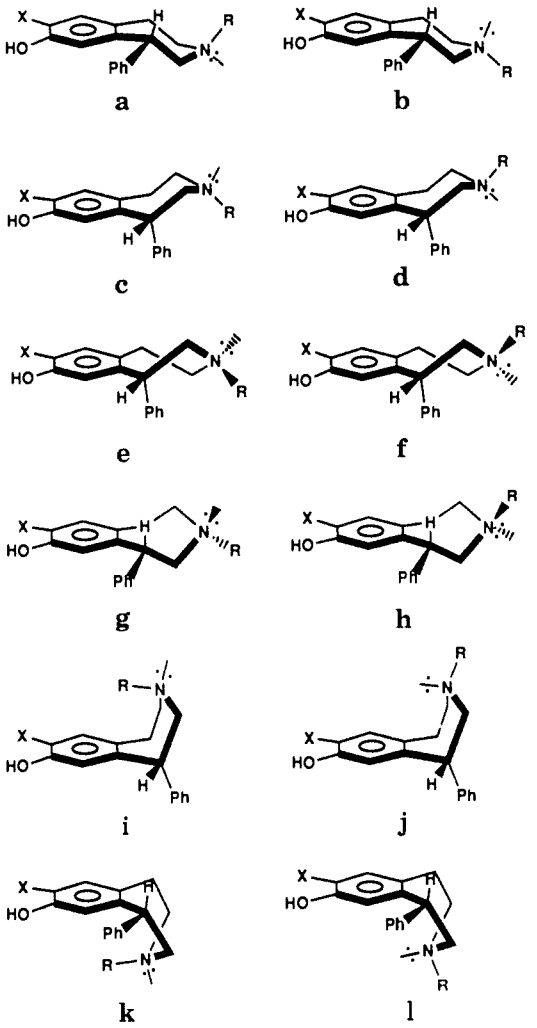

Figure 1. The tetrahydroazepine ring conformations considered in the present study; X = OH, R = H or X = Cl, R = CH_3.

equatorial phenyl group (Figure 1, structures **g** and **h**) in an attempt to understand the D-1 receptor selectivity of 1 and analogues on the basis of calculated molecular electrostatic potentials.[11] The twist conformation was taken from the X-ray structure of the *N,N*-dimethyl quaternary salt analogue of the dimethyl ether of 1.[2]

The optimum angle between the planes of the aromatic rings for efficient binding to the dopamine D-1 receptor has been discussed by Ladd et al.[12] In a study of 2-aryldopamine analogues, which are structurally similar to 1, these authors conclude that the two aromatic rings should approach coplanarity in order to efficiently bind to the D-1 receptor.

Table I. Pharmacological Data for 1–7

compd	[^{3}H]fenoldopam binding, rat striatum: K_i, μM	adelylate cyclase stimulation: ED_{50}, μM or [% above control (μM)]	adelylate cyclase stimulation: EC_{50}, μM or [% of DA response (μM)]
1	0.0051 ± 0.0009[a,b]	0.063[a,b]	0.08[b,c]
2	0.024 ± 0.005[a]	0.43[a]	
3	1.22[a]	[20(10)][a]	
4			inactive, 10[c]
5			inactive, 1[d]
6			[71(1)][d]
7			0.21[d]

[a] Data from ref 10. [b] *R* enantiomer, all other data in this table refer to racemates. [c] Data from ref 14. [d] Data from ref 15.

Table II. Dopamine D-1 Receptor Binding Data for 8–15

compd	config	[^{3}H]SCH 23390 displacement: K_i, nM	compd	config	[^{3}H]SCH 23390 displacement: K_i, nM
8	*R*	0.3,[a] 0.4 ± 0.1[b]	11	rac	6[c]
8	*S*	192[a]	12	rac	0.8[d]
9	6a*S*,13b*R*	1.9 ± 0.6[b]	13	rac	0.4[d]
9	6a*R*,13b*S*	531 ± 178[b]	14	rac	1.6[d]
10	6a*S*,13b*S*	513 ± 57[b]	15	rac	7[e]
10	6a*R*,13b*R*	898 ± 190[b]			

[a] Data from ref 7. [b] Data from ref 20. [c] Data from ref 17. [d] Data from ref 18. [e] Data from ref 19.

The axial/equatorial disposition of an *N*-alkyl group in derivatives of 1 has also been discussed. In order to explain the inactivity of the *N*-*n*-propyl analogue of 1, Nichols[13] argues that an axial *N*-alkyl conformation, and consequently an equatorial unshared electron pair (or NH), may be necessary for D-1 receptor activity. This author assumes that the *N*-*n*-propyl group, in contrast to hydrogen and small alkyl groups, strongly prefers an equatorial position.

Very few studies on the conformational properties of antagonist 8 and its analogues in relation to observed receptor affinities have been reported. After the present work was completed, Berger et al. reported force-field calculations on 8 and some conformationally constrained analogues.[20] They concluded that an axial phenyl substituent is detrimental to D-1 receptor affinity.

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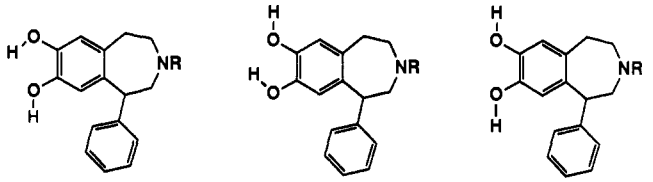
Chart II



I II III

As it is not necessary that the biologically active conformations of agonists and antagonists in the benzazepine series be identical, in the present work we will discuss the analogues of compounds 1 and 8 separately.

Recently, pharmacological and biochemical data for a number of new analogues of 1 and 8, including conformationally constrained ones, have become available.[10,14-20] As previous attempts to identify the biologically active conformations of 1 and 8 have been based on limited studies on a small number of analogues, this prompted us to employ comprehensive molecular mechanics (MM2(85)) calculations of geometries and conformational energies of an extended set of analogues of 1 and 8 in an attempt to identify the biologically active conformations of these compounds with respect to dopamine D-1 receptor agonism and antagonism, respectively. The availability of receptor binding data for active and inactive conformationally constrained compounds is particularly valuable in this context.

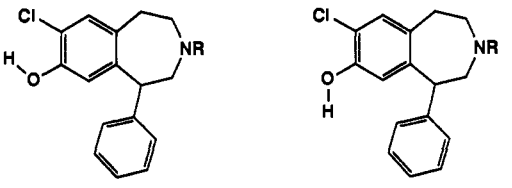
The compounds discussed in the present work are shown in Chart I. The pharmacological and biochemical data used in the discussion are summarized in Tables I and II.

Computational Methods

Conformational energies and energy-minimized molecular geometries were calculated by using the molecular mechanics program MM2(85) developed by Allinger and co-workers.[21-24] Bond order dependent torsional force constants, as described by Liljefors and Allinger,[23] were included in the calculations. Electrostatic interactions involving a phenyl ring[25,26] were calculated with a $C(sp^2)$–H bond dipole of 0.7 D with the negative end at the carbon atom and a $C(sp^2)$–$C(sp^3)$ bond dipole of 1.0 D with the negative end at the sp^2-hybridized carbon atom. These bond dipoles reproduce the dipole moment of toluene and give essentially identical results for phenyl–phenyl interactions as those we have previously reported employing point charges on aromatic carbons and hydrogens.[25] Electrostatic interactions in the MM2(85) program are expressed in terms of dipole–dipole interactions, and the use of bond dipoles instead of point charges for phenyl ring electrostatics is therefore necessary for general calculations.

Hydrogen bonding between hydroxyl groups and between a hydroxyl group and a chlorine atom were included in the calculations according to Allinger et al.[27]

Chart III



IV V

Table III. Results of Molecular Mechanics (MM2(85)) Calculations on 1–4[a]

conformer	1	1-NH^+	2	3	4
a	1.1	0.0	1.4		4.2
b	0.0	0.0	0.4		3.1
c	0.4	0.0		0.0	0.2
d	0.3	0.0		0.7	0.0
e	1.3	2.1	0.0	5.5	0.2
f	2.6	2.1	1.4	4.8	2.2
g	1.7	1.8	3.8	0.3	2.3
h	1.9	1.8	4.7	0.5	2.5
i	2.3	2.0		2.8	1.7
j	2.4	2.0		3.0	2.2
k	4.4	4.2	4.8		4.0
l	4.2	4.2	7.6		4.3

[a] Relative energies are in kcal/mol.

Input structures for the MM2(85) calculations were constructed by using the molecular modeling program MIMIC.[28,29]

Most of the energy calculations were done on the unprotonated amines (with the unshared electron pair represented by a pseudoatom). The energies for different conformations of compounds 1 and 8 were also calculated for the N-protonated molecules with the appropriate nitrogen atom type available in MM2(85). Note that in using this atom type in MM2(85), only the "steric" interactions of the protonated amino group are taken into account. The MM2(85) program does not treat monopole–dipole interactions, and thus electrostatic interactions due to the ammonium-type nitrogen are not included.

Results

The tetrahydroazepine ring conformations **a**–**l** considered in this work are shown in Figure 1. In addition, the three dihydroxy conformations I–III shown in Chart II have been considered for 1. This was done in order to investigate the influence of hydroxy group conformation on the conformational properties of the tetrahydroazepine ring.

Energy minimizations of conformers **a**–**l** of 1 using each of the dihydroxy conformations I–III display only a very small sensitivity of the relative conformational energies of the tetrahydroazepine ring conformations to the dihydroxy conformation. For this reason, only the lowest energy dihydroxy conformation I was used in the subsequent calculations on **2**–**7**. Conformation I is calculated to be on the average 0.3 and 3.5 kcal/mol lower in energy than conformations II and III, respectively.

For the 7-chloro analogue of 1 two conformations of the hydroxy group, IV and V (Chart III), were investigated.

Also in this case, the relative conformational energies of conformers **a**–**l** are essentially insensitive to the hydroxy group conformation. In the calculations on **8**–**15** discussed below the lowest energy conformation IV has been employed. This conformation is calculated to be on the average 1.4 kcal/mol lower in energy than conformation V.

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Conformational Analysis of the Tetrahydroazepine Ring in 1–7. The results of the MM2(85) calculations on 1–4 are given in Table III. The relative energies of conformers **a–l** (Figure 1) of **5–7** are essentially identical with those of 1 and are therefore not reported in the table.

The lowest energy minimum of 1 is calculated to be a chair conformation of the tetrahydroazepine ring with the phenyl ring and the (N)H atom in equatorial positions, conformation **b** in Figure 1. However, the chair conformation with an axial phenyl ring, **1c/d**, is calculated to be less stable than **1b** by only 0.3–0.4 kcal/mol. These results are in agreement with previous force-field calculations on fenoldopam (SK&F 82526), a 6-chloro-4′-hydroxy derivative of 1, for which energy differences between chair conformers ranging from 0.19 to 0.58 kcal/mol, in favor of an equatorial phenyl ring, have been calculated.[15] The conformation of the *R* enantiomer of fenoldopam (hydrobromide salt) in the crystal structure is identical with the calculated lowest energy conformer of 1.[15]

The twist and boat conformers of 1 are calculated to be higher in energy than the chair conformer **1b** by 1.3–2.6 and 2.3–4.4 kcal/mol, respectively (Table III).

The relative energies of axial and equatorial NH depend on the tetrahydroazepine ring conformation and on the disposition of the phenyl ring. Only in **1a** is the energy for an axial (N)H atom calculated to be significantly different from the energy of the corresponding equatorial one **1b**. In conformer **1c** the axial (N)H atom interacts via attractive electrostatic interactions with the axial phenyl ring. This lowers the energy of **1c** in relation to **1d** and offsets the expected higher energy of the axial (N)H position. The same type of stabilization is present in conformer **1e**. In the boat conformers **1i** and **1k** the axial (N)H atom is stabilized by electrostatic interactions with the catechol ring.

The results of the calculations on N-protonated 1 are shown in Table III. The calculated relative conformational energies are very similar to those for the unprotonated case. The chair conformers are the most stable ones, and equatorial and axial phenyl rings are calculated to be of equal energy. The twist and boat conformers are 1.8–4.2 kcal/mol higher in energy. Thus, the conformational properties of the tetrahydroazepine ring of 1 do not seem significantly influenced by protonation of the nitrogen atom.

The conformationally constrained **2** cannot adopt the chair conformation with an axial phenyl ring, **2c/d**, and the boat conformation **2i/j**. The lowest energy is found for the twist conformer **2e** with an axial phenyl group (Table III). The chair conformer **2b** with an equatorial phenyl ring and (N)H, corresponding to the calculated lowest energy minimum for 1, is only 0.4 kcal/mol higher in energy than **2e**. This is in agreement with reported results from force-field calculations on a dehydroxy analogue of **2**, which give a calculated energy difference between the corresponding twist and chair conformers of 0.19 kcal/mol, favoring the twist conformer.[10] NMR studies in solution indicate that **2** adopts a chair conformation with an equatorial phenyl ring (**2b**).[10]

The chair conformation **3a/b** with an equatorial phenyl group and the boat conformation **3k/l** cannot be attained by **3**. The chair conformer **3c** with an axial phenyl ring is calculated to be of lowest energy for this compound (Table III). This conformer is observed in the X-ray structure of the 6,7-dimethoxy derivative of **3** (hydrochloride) and by NMR studies in solution.[10] The twist conformer **3e** corresponding to the lowest energy structure of compound **2** is calculated to be of high energy for compound **3**. The twist conformers **g** and **h**, with equatorial phenyl rings, are both of low energy for **3**, but of high energy for compound **2**.

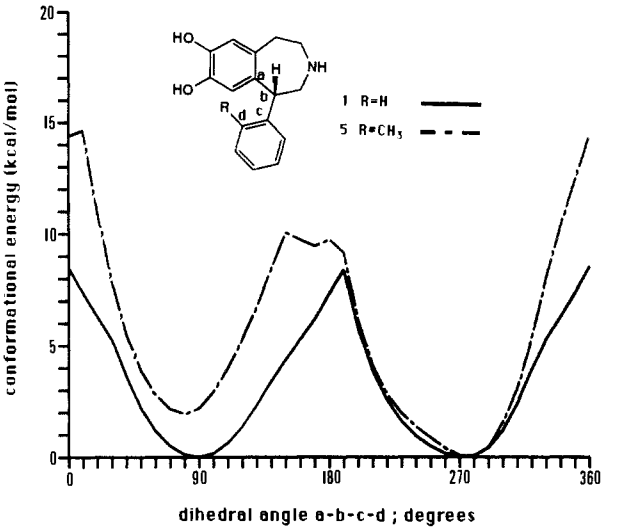

Figure 2. Calculated potential energy curves for rotation about the bond connecting the phenyl ring and the tetrahydroazepine ring in compounds 1 and 5. The dihedral angles refer to the *R* configuration.

The calculated lowest energy minimum for **4** is a chair conformer with an axial phenyl ring (**4d**, Table III). Conformer **4c**, with an axial (N)H and the twist conformer **4e** are only slightly higher in energy. Note that conformer **4b**, which corresponds to the most stable conformer of the parent 1, is of high energy. Repulsive van der Waals interactions between the 9-methyl group and the phenyl ring in **4b** considerably increase the energy of this conformer compared to that of **1b**.

Phenyl Group Orientations in Compound 1 and Analogues. As mentioned in the introduction, it has been argued that a productive binding to the dopamine D-1 receptor may require that the aromatic rings in compounds 1–7 approach coplanarity.[12] In order to probe this suggestion, potential energy curves for rotation about the bond connecting the phenyl ring and tetrahydroazepine ring were calculated for 1 and **5**. The corresponding potential energy curves for **6** and **7** (not shown) are essentially identical with the curve for 1.

As coplanarity of the aromatic ring planes requires an equatorial disposition of the phenyl ring, the calculations were performed on conformer **b** (Figure 1), which is the lowest energy conformer of this type for 1 and **5**. The calculated potential energy curves are shown in Figure 2. Energy minima are found for dihedral angles of 80–90° and 270–280°, corresponding to approximately orthogonal phenyl and catechol ring planes. These minima are energetically degenerate in 1, while in **5** the minimum at about 80° is about 2 kcal/mol higher in energy than the minimum at about 270°. The reason for the higher energy of the conformation of **5** with a dihedral angle of about 80° is steric repulsive interactions between the *o*-methyl group and methylene hydrogens on the tetrahydroazepine ring. The calculated preferred orientation of the phenyl ring in **1b** is in complete agreement with the observed orientation of this ring in the crystal structure of fenoldopam.[15]

The energy barriers between the minima are calculated to be high for both molecules, at least 8–9 kcal/mol, depending on the interconversion pathway. Coplanarity of the phenyl and the catechol ring planes is achieved at dihedral angles of 0° and 180°. The conformational energies at these dihedral angles are substantial. For 1 they

Table IV. Results of Molecular Mechanics (MM2(85)) Calculations on 8–10 and 15[a]

conformer	8	8-NH$^+$	9	10	15
a	3.7	3.5	1.7	2.2	4.0
b	0.7	0.8	0.0	0.5	0.0
c	4.2	4.6		4.5	
d	0.0	0.0		0.0	
e	3.3	3.9		4.0	
f	3.4	3.2		1.3	
g	3.0	3.3	5.1	7.2	5.3
h	3.0	3.2	4.7	6.4	8.7
i	5.6	6.0	7.0	5.8	8.7
j	3.3	3.0	5.9	1.9	10.0
k	9.2	9.5	5.9	4.0	5.3
l	4.3	3.4	4.3	1.2	9.1

[a] Relative energies are in kcal/mol.

are calculated to be 8.5 kcal/mol, and for **5** the energies are 14.5 and 9.6 kcal/mol, respectively.

Conformational Analysis of the Tetrahydroazepine Ring in 8–15. The results of the molecular mechanics calculations on **8–10** and **15** are shown in Table IV. The calculated relative conformational energies for **11–14** are essentially the same as those for **8** and are not shown. As was the case for **1** (see above), the results of the calculations on the N-protonated **8** are very similar to those for the unprotonated molecule.

The lowest energy minimum for **8** is calculated to be a chair conformer with an axial phenyl ring and an equatorial *N*-methyl group, conformer **d** in Figure 1. However, the chair conformer **b** with an equatorial phenyl ring and *N*-methyl group, corresponding to the preferred conformation for **1**, is only 0.7 kcal/mol higher in energy. All other conformers are at least 3.0 kcal/mol higher in energy than **8d**. These results are in agreement with calculations reported by Berger et al.[20] The solid-state conformation of **8**, as its maleate, corresponds to **8b**.[20] The conformations with an axial *N*-methyl group are calculated to be 2.3–4.9 kcal/mol higher in energy than the corresponding conformations with an equatorial one. Thus, the N-methylated tetrahydroazepine ring displays significantly less conformational flexibility than the *N*-unsubstituted derivative **1** (Table III).

The calculated results for the conformationally constrained analogue **9** show that conformer **b**, a chair with an equatorial phenyl ring and *N*-methyl group, is the most stable one (Table IV). An axial *N*-methyl group increases the energy by 1.7 kcal/mol. Due to conformational constraints, conformers **c–f** cannot be adopted by this compound. The remaining twist and boat conformers have high calculated conformational energies, 4.3 kcal/mol or higher. Conformational analysis by Berger et al. gave as a result a single low-energy conformation of **9** corresponding to **9b**.[20]

The lowest energy minimum for the cis analogue **10** is the same as that for compound **8**, a chair conformation with an axial phenyl ring and an equatorial *N*-methyl group (**10d**, Table IV). The chair conformation **10b** which corresponds to the most stable conformer for compound **9** is only 0.5 kcal/mol higher in energy.

Compound **15** cannot adopt the chair conformations **c** and **d** and the twist conformations **e** and **f**. The chair conformer **1b** (equatorial phenyl group and *N*-methyl) is the calculated lowest energy one (Table IV). All other conformations of **15** are calculated to be higher in energy by at least 4 kcal/mol.

Axial–Equatorial Energy Differences for *N*-Alkyls. As mentioned in the introduction, it has been suggested that an axial *N*-alkyl group may be required for D-1 activity and that the axial–equatorial energy difference of an *N*-*n*-propyl group may rationalize the inactivity of compounds with this substituent.[13] To examine the validity of this argument we have calculated the axial–equatorial energy differences for the *N*-ethyl and *N*-*n*-propyl derivatives of **8a/b**. The results are shown in Table V, which also includes the results for **8a/b** and for its N-unsubstituted derivative. As the substituent in the 8-position does not significantly influence the results of these calculation, the data in Table V are also valid for the corresponding derivatives of **1a/b**.

Table V. Energy Differences between Axial and Equatorial N-Substituents (in kcal/mol) for Derivatives of **8** in a Chair Conformation with an Equatorial Phenyl Group

NH	*N*-methyl	*N*-ethyl	*N*-*n*-propyl
1.1	3.0	2.4	2.5

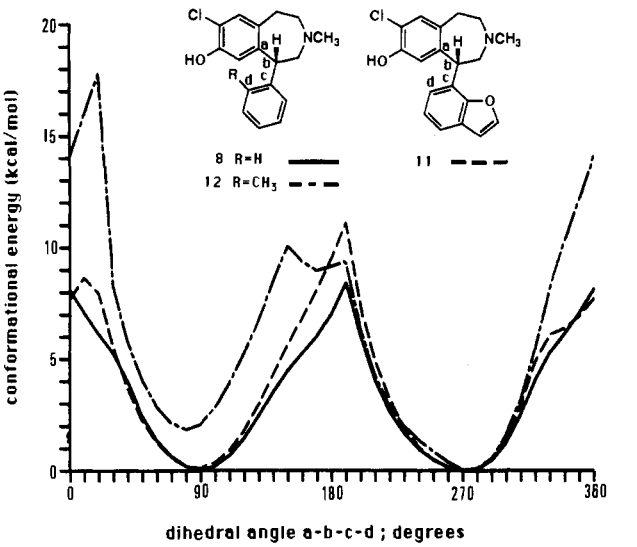

Figure 3. Calculated potential energy curves for rotation about the bond connecting the phenyl ring or benzofuran ring and the tetrahydroazepine ring in compounds **8**, **11**, and **12**. The dihedral angles refer to the *R* configuration.

An *N*-alkyl substituent is calculated to increase the axial–equatorial energy difference by 1.3–1.9 kcal/mol compared to that of the N-unsubstituted case (Table V). However, this energy increase is calculated to be very similar for methyl, ethyl, and *n*-propyl. Thus, according to our calculations, the suggestion made by Nichols[13] that the *n*-propyl group should more strongly prefer an equatorial position compared to smaller alkyl groups is unfounded.

Phenyl Group Orientations in Compound 8 and Analogues. The potential energy curves for rotation about the bond connecting the phenyl ring, benzofuran ring, or *o*-methylphenyl ring in compounds **8**, **11**, and **12** and the tetrahydroazepine skeleton are shown in Figure 3. The tetrahydroazepine conformer **b** (Figure 1) was employed in these calculations. The corresponding potential energy curves for **13** and **14** are essentially identical with that of **8** and are therefore not shown.

Compound **8** is calculated to have two degenerate energy minima at dihedral angles of about 90° and 270°, respectively. The potential energy curve for **8** is virtually identical with that of **1** (cf. Figures 2 and 3). The shapes of the energy curves for **8** and **11** are similar. Thus, the annelated furan ring in **11** does not significantly reduce the flexibility of the phenyl ring in the vicinity of the two energy minima. However, at a dihedral angle of 180° the energy of compound **11** is calculated to be 2.2 kcal/mol higher than that of **8**. The calculated curve for **12** is very

similar to that calculated for **5** (cf. Figures 2 and 3). Thus, the *N*-methyl group does not significantly influence the calculated potential energy curve.

The calculated dihedral angles (as defined in Figure 3) for the conformationally constrained **9**, **10**, and **15** in conformation **b** are 299°, 270°, and 309°, respectively. Thus, the orientation of the constrained phenyl ring in **10** is almost identical with the preferred orientation of the corresponding phenyl ring in the parent compound **8** (273°). The orientations of the phenyl rings in **9** and **15** deviate by 26° and 36°, respectively, from the preferred one in **8**.

Discussion

In the following, the conformational analysis of **1–7** and **8–15** described above will be discussed in relation to the pharmacological and biochemical data given in Tables I and II in an attempt to identify the biologically active conformations of the parent compounds **1** and **8**. In each of the two series of compounds the lipophilicities should be similar. Furthermore, structural similarity justifies the assumption that the compounds in each series have the same binding mode in the receptor. Thus, in the absence of repulsive steric interactions with the receptor, the relative affinities/activities of the compounds studied should be largely determined by differences in conformational energies of the receptor-bound conformation. (Thermodynamically, an increase of the conformational free energy by 1.4 kcal/mol at 300 K corresponds to a decrease in the affinity, as measured by K_i, by a factor of 10.)

We are discussing possible biologically active conformations in terms of the 12 local energy minima **a–l** in Figure 1. In this discussion we are aware that a biologically active conformation may not necessarily be a local energy minimum for the unbound molecule. However, we believe that the conformations considered in this work represent a sufficiently large part of the low-energy conformational space of the tetrahydroazepine ring to justify this procedure. Small structural distortions of the structures corresponding to the local energy minima in Figure 1 are of course energetically feasible.

Compounds **1** and **8** are highly enantioselective with respect to dopamine D-1 receptor affinity/activity, and the pharmacological/biochemical data for the racemates of these compounds are close to those for the active *R* enantiomers.[2-7] For most of the analogues in Scheme I only the racemates have been studied. We assume that the active isomers of these analogues display the same level of enantioselectivity as **1** and **8** and that the data for the racemates given in Tables I and II are similar to those for the enantiomer which is homochiral with the active *R* configuration of **1** and **8**.

Compounds 1–7, Conformational Energies vs Biological Affinities/Activities. As mentioned in the introduction, Dandridge et al.[8] have suggested that the biologically active conformation of compound **1** is a chair conformation with an axial phenyl ring (**1c/d**, Figure 1). However, on comparing the results of the conformational analysis of compounds **1–4** (Table III) with the data in Table I, it is clear that such a conformation most probably does not correspond to the receptor-bound conformation of **1**. The conformations **c** and **d** cannot be adopted by the active compound **2**, as previously noted by Weinstock et al.[10] Furthermore, the conformational energies of the **c** and **d** conformers of **3** and **4** are calculated to be low. If the suggestion made by Dandridge et al. was correct, this would imply that compounds **3** and **4** should display high activities. However, the affinity of compound **3** is more than 2000 times lower than that of **1**, and compound **4** is inactive in the adenylate cyclase assay (Table I).

The twist conformation **g/h** is observed in the crystal structure of the *N,N*-dimethyl quaternary salt analogue of the dimethyl ether of **1**.[2] Van de Waterbeemd et al. used this conformation as a biologically active one in an attempt to find relationships between calculated molecular electrostatic potentials and pharmacological data.[8] Compound **3** is calculated to have low conformational energies for the twist conformers **g** and **h**. If any of these conformations is the biologically active one, **3** should exhibit a higher biological activity (affinity) than compounds **1** and **2**, which have significantly higher conformational energies for these conformers. As this is not the case (Table I), conformations **g** and **h** cannot be biologically relevant. We have calculated the conformational energies of the *N,N*-dimethyl analogue of **1**. With this substitution pattern on nitrogen, the twist conformation **g/h** is calculated to be only 0.4 kcal/mol higher in energy than the lowest energy one, which in this case, as for **1**-NH^+ (Table III), is calculated to be a chair conformation with an equatorial phenyl ring (**a/b**). The energy for N-protonated **1g/h** is calculated to be 1.8 kcal/mol higher than that for N-protonated **1a/b** (Table III). Thus, the dimethyl substitution on nitrogen significantly lowers the relative conformational energy of the twist conformation **g/h**. The conformation observed in the crystal structure of the *N,N*-dimethyl quaternary salt analogue of the dimethyl ether of **1**[2] is clearly a result of the nitrogen substitution pattern.

The twist conformations **e/f** and the boat conformations **i–l** are not likely candidates for a biologically active conformation for the following reasons. Calculated conformational energies for the twist conformations **e** and **f** indicate that if one of these conformations were the active one, **2** would be significantly more active than **1**. However, **1** has a 5-fold higher affinity ([^{3}H]fenoldopam binding) and a 7-fold lower ED_{50} value in the adenylate cyclase assay than **2** (Table I). Furthermore, with **e** or **f** as the active conformer, the calculated energies in Table III imply that compound **4** should be more active than compound **1** (conformation **e**) or show comparable activity (conformation **f**). As shown in Table I, **4** is inactive.

The boat conformations **i** and **j** cannot be adopted by the highly active **2**. Thus, these conformations may be excluded as probable active ones. The boat conformations **k** and **l** can be excluded, since the high energies of these conformations for compounds **1** and **2** are not compatible with the high affinities/activities of these compounds. Furthermore, if **k** or **l** is the active conformation, compound **4**, which is inactive, should have a biological activity at least as high as those of **1** and **2**.

Only conformations **a** and **b**, with the tetrahydroazepine ring in a chair conformation and with an equatorial phenyl ring, fulfill the requirements for a biologically active conformation. The calculated energies for these conformers are low for the highly active **1** and **2** and high for the poorly active or inactive **3** and **4**. Thus, we conclude that the most probable biologically active conformation of **1** (and its analogues) is a chair conformation with an equatorial phenyl ring, as shown in Figure 4.

It is likely that **1** interacts with the receptor via a hydrogen bond to the nitrogen atom. This may be accomplished by the protonated **1** as a donor or by the unprotonated molecule as an acceptor. In either case the receptor-bound conformations **a** and **b** should be essentially energetically degenerate and the question of whether conformation **a** or **b** is the most probable one for the receptor-bound molecule becomes meaningless.

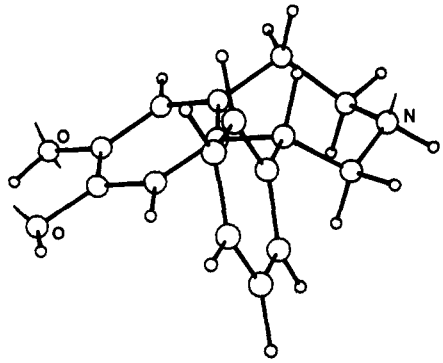

Figure 4. Proposed biologically active conformation for compound 1 with respect to the conformation of the tetrahydroazepine ring and the phenyl ring position. The phenyl ring rotamer shown is the lowest energy one.

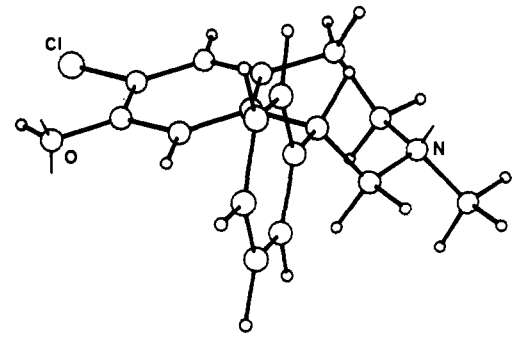

Figure 5. Proposed biologically active conformation for compound 8 with respect to the conformation of the tetrahydroazepine ring and the phenyl ring position. The phenyl ring rotamer shown is the lowest energy one.

The inactivity of **5** (Table I) may be rationalized in two ways. Either the *o*-methyl group prevents a necessary coplanarity between the two aromatic rings, as discussed by Ladd et al.,[12] or the inactivity is due to steric repulsive interactions between the *o*-methyl group and the receptor. The calculated potential energy curves for rotation about the bond connecting the phenyl ring and the seven-membered ring are very similar between 190° and 280° for compounds **1** and **5** (Figure 2). The energy increase due to phenyl ring reorientation from the most stable orientation to one that gives approximately orthogonal aromatic ring planes is essentially identical for the two compounds. Thus, the argument that **5** is inactive because it cannot achieve a correct phenyl group orientation is not valid. The inactivity of **5** is most probably due to repulsive steric interaction between the *o*-methyl group and the receptor.

The *p*-methyl group in **7** is very well accommodated by the receptor cavity, as evidenced by its high activity in the adenylate cyclase activity (Table I). Unfortunately, no receptor binding data are available for **5–7**. The high affinities of the analogous **12–14** (Table II) make it possible that **5–7** have good receptor affinities, but **5** and **6** have low efficacies.

The calculated potential energy curves in Figure 2 indicate that it is not likely that the aromatic rings in **1** are coplanar in the receptor-bound molecule. Such a phenyl ring orientation implies an energy penalty of at least 6–7 kcal/mol, which in our opinion is prohibitively high. However, a phenyl ring orientation corresponding to a dihedral angle deviation from the preferred one by up to 30° may be feasible.

Compounds 8–15, Conformational Energies vs D-1 Receptor Affinities. An analysis of the affinity data in Table II and the calculated conformational energies in Table IV strongly indicates that the biologically active conformation for the D-1 receptor antagonist **8** and its analogues is a chair conformation with an equatorial phenyl ring and an equatorial *N*-methyl group (conformer **b** in Figure 1). In other words, it is the same tetrahydroazepine ring conformation as concluded above to be the biologically active conformation for the agonist **1**. This supports the conclusion drawn by Berger et al. on the basis of force-field calculations on compounds **8–10**.[20] The arguments for our conclusion with respect to **8** are as follows.

It is obvious that conformations **c–f** can be excluded as candidates for the biologically active conformation of **8** and its analogues. These conformations cannot be adopted by the highly active compounds **9** and **15**. The calculated energies for conformations **g–l** are high for comopunds **8**, **9**, and **15**. In our opinion, a biologically active conformation with such a high conformational energy is not compatible with the observed high affinity of these compounds.

The chair conformation **a** with an axial *N*-methyl group is not a likely candidate for the biologically active conformation. The calculated conformational energy of **8a** is quite high, and with **a** as the biologically active conformation the significantly lower energy of compound **9** implies that this compound should display a higher affinity than **8**, in contradiction with experimental data.

The calculated conformational energies for the chair conformation **b**, with an equatorial phenyl group and *N*-methyl group, fit very well the receptor binding data for compounds **8**, **9**, and **15** in Table II and also those for compounds **11–14**, for which the relative conformational energies are essentially identical with those of **8**. The calculated energies of the **b** conformation of the high-affinity compounds **8**, **9**, and **11–15** are all within 0.7 kcal/mol of the lowest energy minimum. No other conformation in Table IV displays low energies for all the high affinity compounds. The energy-minimized structure of **8b** is shown in Figure 5.

The very low affinity of compound **10** cannot be rationalized by the calculated conformational energies or by an "incorrect" phenyl ring orientation. There is no conformation in Table IV which is of high energy for **10** and of low energy for **8**, **9**, and **15**. The phenyl ring orientation in **10** is very similar to the preferred one for **8** (see above). The most probable reason for the low affinity of **10** is repulsive steric interactions between the ethano bridge and the receptor. This conclusion implies that **11** and **12**, in order to avoid such repulsive interactions, should bind to the receptor with the *o*-methyl group and the furan ring, respectively, pointing in the same direction as the nitrogen lone pair (or NH).

As was concluded for **1** and its analogues, it is not likely that the phenyl ring in the receptor-bound conformation of **8** and its analogues with unconstrained phenyl rings deviates by more than about 30° from the preferred orientation. The potential energy curve for phenyl ring reorientation in **8** (Figure 3) shows that for dihedral angles significantly outside this range, the conformational energy becomes prohibitively high for a high-affinity compound. However, the phenyl ring orientations in the constrained analogues **9** and **15** show that dihedral angle deviations from the preferred one in **8** by at least 36° (the calculated value for **15**, see above) are compatible with high affinity. For the constrained analogues there is of course no conformational energy penalty involved.

Conclusions

Conformational analysis in conjunction with experimental receptor binding data, including data for conformationally constrained active and inactive compounds,

strongly indicate that the most probable biologically active conformation for the D-1 selective dopamine receptor agonist 1, as well as for the antagonist 8, is a chair conformation (or a conformation structurally close to it) with an equatorial phenyl ring. In both cases it is concluded that the orientation of the phenyl ring in the receptor-bound molecule does not deviate in terms of dihedral angles by more than about 30° from the preferred orientation. The *N*-methyl group in compound 8 most probably has an equatorial position in the active conformation.

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Synthesis and Biological Evaluation of 4-Fluoro-, 7-Fluoro-, and 4,7-Difluoro-5,6-dihydroxytryptamines

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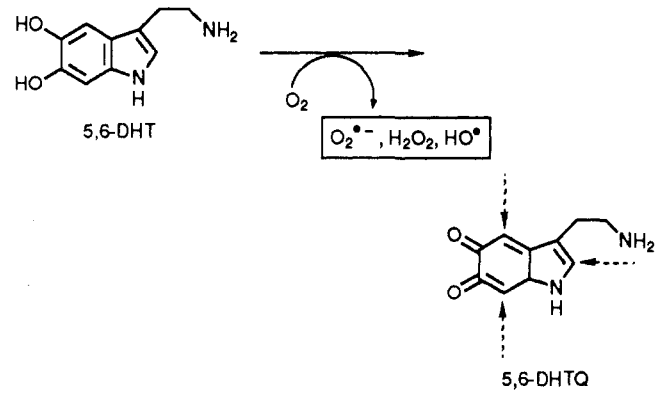
The 5,6-dihydroxytryptamine (5,6-DHT) derivatives 4-fluoro- and 7-fluoro-5,6-DHTs (**26a,b**) and 4,7-difluoro-5,6-DHT (**26c**) were synthesized from 3-fluoroanisole (1) and 1,4-difluoro-2,3-dimethoxybenzene (**13**), respectively. Efficient methods were developed for the conversion of 1 to 4-fluoro- and 7-fluoro-5,6-bis(benzyloxy)indoles (**12a,b**, respectively) and **13** to 4,7-difluoro-5,6-[(diphenylmethylene)dioxy]indole (**19**) via reductive cyclization of 2-nitro-β-(dialkylamino)styrenes prepared in situ from 2-nitrotoluenes. Indoles **12a,b** and **19** were then converted to **26a–c** via the corresponding indole-3-acetonitriles. The fluorine-substituted 5,6-DHTs displayed increased phenol acidities, determined spectrophotometrically, and decreased inherent potential to undergo oxidation as determined by cyclic voltammetry. Fluorine substitution did not have a significant adverse effect on the cytotoxic potential as judged from the IC_{50} values of 117, 125, 135, and 92 μM for **26a,c** and 5,6-DHT, respectively, for the inhibition of incorporation of [^{3}H]thymidine into the DNA of neuroblastoma clone N-2a cells in culture. Surprisingly, **26a–c** exhibited 32-, 23-, and 13-fold higher affinities, respectively, compared to 5,6-DHT for the serotonergic uptake system of N-2a cells as measured by the ability of **26a–c** and 5,6-DHT to antagonize the uptake of [^{3}H]5-HT into the N-2a cells. These desirable chemical and biological properties of **26a–c** should make them useful tools for the study of the molecular mechanism of neurodegenerative action of 5,6-DHT.

5,6-Dihydroxytryptamine (5,6-DHT, Chart I) is a general pharmacological tool used to produce selective destruction of 5-hydroxytryptamine (5-HT) containing nerve terminals.[1,2] It is selective due to its high-affinity, active uptake by the serotonergic membrane pumps. The neurodegeneration is believed to be initiated by the alkylation and free-radical-induced damage of essential neuronal constituents by the electrophilic quinones and the reduced O_2 species such as H_2O_2, $O_2^{\bullet-}$ and HO•, respectively, produced by the intraneuronal autoxidation of 5,6-DHT.[2-4] Because of the complexity of the autoxidation reaction, it has not yet been possible to characterize the DHT-derived product(s), postulated to be 5,6-DHTQ (Chart I). With radiolabeled 5,6-DHT, it has been shown that the DHT-derived autoxidation products undergo extensive covalent binding with protein nucleophiles both in vitro[4] and in vivo.[5] However, the nature of this protein–quinone interaction, including the relative importance of the postulated electrophilic sites of 5,6-DHTQ toward alkylation, remains to be determined. It was thought that if the unsubstituted positions in the 5,6-DHT ring were independently or simultaneously blocked, it might be possible to determine the relative importance not only of the putative electrophilic sites but also of the alkylation and free-radical-induced damage in neurodegeneration. Previously, we designed and synthesized 4-methyl-, 7-methyl-, and 4,7-dimethyl-5,7-DHTs for this purpose.[6,7] The methyl-substituted analogues suffered from two drawbacks. First, they underwent much more rapid autoxidation than 5,6-DHT, making them difficult to handle during in vivo studies. In addition, their affinity of uptake by the serotonergic membrane pumps was significantly

Chart I[a]



[a] The broken arrows point to putative sites of alkylation reactions.

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